

Supplementary Table II. Degenerate codon library design for structurally targeted mutagenesis and isolated BFP variants

Position	61	64	145	148	150	163	165	167	203	205	220	224	Rel. Fl. ^a
Design ^b	DYA	TTK	KBC	CAC	DTA	RYG	TWC	ATK	RSC	KCA	MTG	RBG	
BFPwt	V	F	F	H	V	V	F	I	T	S	L	V	1.0 ± 0.3
BFP.A5		L	A		I	A						R	5.4 ± 0.6
BFP.B3		L			I							R	4.9 ± 0.3
BFP.A4		L			I							R	4.6 ± 0.3
BFP.A2		L			I					A		R	3.8 ± 0.1
BFP.B6		L										R	3.7 ± 0.2
BFP.A8		L			I	A						R	3.4 ± 0.3
BFP.A9	I	L			I							R	2.5 ± 0.2
BFP.A12	T	L			I	A				A		R	2.2 ± 0.1
BFP.B7	I	L	L		I	A				A		R	2.0 ± 0.1
BFP.A10		L				A						R	2.0 ± 0.3
BFP.B4		L	V		I	T				A		R	1.9 ± 0.2
BFP.A7		L	C			A						R	1.9 ± 0.2
BFP.B8		L											1.6 ± 0.3
BFP.A6	T	L											1.4 ± 0.2
BFP.B5					L								1.4 ± 0.5
BFP.A11	T	L			I	A							1.4 ± 0.2
BFP.A1		L			I	A		M					1.1 ± 0.2

^a Ranked by mean relative whole-cell fluorescence as measured via fluorimetry. ^bIUPAC nomenclature for degenerate codons is as follows: B(CGT), D(AGT), K(GT), R(AG), S(CG), W(AT), Y(CT), M(AC), (See also Supplementary Table I)